# Differential Introduction of DNA Damage and Repair in Mammalian Genes Transcribed by RNA Polymerases I and II

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We have developed a general quantitative method for comparing the levels of drug-induced DNA crosslinking in specific mammalian genes. We observed a dramatic difference between the efficiency of the removal of both psoralen monoadducts and interstrand crosslinks from the rRNA genes and the efficiency of their removal from the dihydrofolate reductase (DHFR) gene in cultured human and hamster cells. While 90% of the interstrand crosslinks were removed from the human DHFR gene in 48 h, less than 25% repair occurred in the rRNA genes. Similarly, in Chinese hamster ovary cells, 85% repair of interstrand crosslinks occurred within 8 h in the DHFR gene versus only 20% repair in the rRNA genes. The preferential repair of the DHFR gene relative to that of the rRNA genes was also observed for psoralen monoadducts in cells from both mammalian species. In human-mouse hybrid cells, the active mouse rRNA genes were five times more susceptible to psoralen modification than are the silent rRNA human genes, but adduct removal was similarly inefficient for both classes. We conclude that the repair of chemical damage such as psoralen photoadducts in an expressed mammalian gene may depend upon the class of transcription to which it belongs.

Environmental stresses such as radiation or chemical carcinogens that damage DNA can initiate specific genetic alterations, from simple point mutations to complex chromosomal rearrangements (29). Similar structural alterations have been correlated with severe human hereditary syndromes (28), with inherited predisposition to cancer (12), and with somatically acquired tumorigenicity (1). Moreover, recent studies indicate a correlation between the location of chromosomal fragile sites and the genomic targets of certain carcinogens in mammalian cells (38). It is thus important to understand the mechanism(s) by which DNA damage might affect the stability of expressed, or potentially expressible, genetic information in mammalian cells.

To respond to the deleterious actions of environmental DNA-damaging agents, mammalian cells, like the cells of most other living organisms, have evolved the capacity to recognize and remove lesions from their DNA. The importance of these DNA excision-repair processes is dramatically illustrated by the human hereditary disease xeroderma pigmentosum, in which there is a deficiency in repair (5); individuals afflicted by this syndrome are unusually sensitive to the mutagenic actions of carcinogens (18), and they exhibit increased frequencies of neoplasia (15). Although several human genes that complement excision-repair deficiencies in mutant rodent cells have been recently cloned, their precise functions are still unknown (30, 34). Moreover, the recent detection of excision-repair activities that act on purified DNA in extracts from human cells has not yet defined the precise molecular steps of this process (35). Thus, many of the details of excision-repair have yet to be characterized in mammalian cells.

A major focus has been to understand the mechanism(s) by which the DNA repair machinery can recognize and access DNA damage that is deeply embedded in the complex structures of chromatin. A particularly intriguing problem

has been to establish the relationship between DNA repair

and transcription. Bulky DNA lesions in mammalian cells

can interfere with transcription (23). Following the analysis

As a DNA damaging agent, we used the photocatalyzed reaction of the psoralen derivative 4'-hydroxymethyl-4,5',8-trimethyl psoralen (HMT), whose molecular specificity and details of reaction with DNA are well characterized (4, 31). Psoralens are naturally occurring tricyclic aromatic compounds that intercalate into DNA and form covalent adducts with pyrimidine bases when exposed to near ultraviolet light (UVA). They possess groups that react specifically with carbon positions 5 and 6 of pyrimidines, allowing covalent

of repair in the dihydrofolate reductase (DHFR) gene of UV-induced pyrimidine dimers (3) and of psoralen DNA photoadducts (32), two distinct mechanisms for the restoration of transcription in lesion-containing mammalian genes were proposed, selective repair of transcribing DNA strands (20) and translesion replication (31). In the first mechanism, removal of UV-induced pyrimidine dimers occurs preferentially in the transcribed strand (i.e., the template for the RNA polymerase). In the second mechanism, replication of DNA carrying transcription-blocking lesions generates undamaged genes in daughter chromatids; translesion replication may be particularly important in restoring transcription competency for unrepaired lesions such as bulky chemical adducts (33). Preferential repair of DNA damage from actively transcribed sequences has been documented in a variety of mammalian genes as well as genes of other species (26). A common characteristic of these genes is that they all are transcribed by RNA polymerase II (24). Are there any actively transcribed genes that are not preferentially repaired? rRNA genes form a distinct category of housekeeping genes in that they are transcribed by a distinct RNA polymerase, RNA polymerase I (pol I) (24). To establish the possible generality of preferential repair, we have compared the repair of DNA damage in genes which are transcribed by two different mammalian RNA polymerases, pol I and RNA polymerase II (pol II).

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addition on either strand of the DNA (monoadducts) or on both strands of the DNA (interstrand crosslinks). Modifying our recently developed procedure (31), we are able to simultaneously detect psoralen adducts induced by HMT and UVA in two housekeeping genes: the pol I-transcribed rRNA genes and the pol II-transcribed DHFR gene. We report that the removal of psoralen adducts in human fibroblasts is much more rapid in the DHFR than in the rRNA gene. Such preferential repair of these pol II- versus pol I-transcribed genes is also obtained in Chinese hamster fibroblasts, suggesting the possible generality of this observation. In human-mouse cell hybrids, the repair inefficiency of the adducts in the pol I-transcribed genes appears to be independent of their transcriptional state. We conclude that preferential DNA repair of psoralen adducts may depend upon transcription by pol II.

#### **MATERIALS AND METHODS**

Cell cultures. The human cell line VA2-6A3 (obtained from G. Attardi, California Institute of Technology, Pasadena); a Chinese hamster ovary (CHO) cell line, B11 (obtained from R. T. Schimke, Department of Biological Sciences, Stanford University, Stanford, Calif.); and the swiss mouse 3T3 fibroblasts and human HT-1080 fibroblasts were grown as described (17, 20, 32). The hybrid lines 6-F1 (56-05 F1) and 12-F8 (55-84 F8) (obtained from K. Huebner, The Wistar Institute, Philadelphia, Pa.) originated from the fusion of HT-1080 cells with a derivative of 3T3 cells and from the fusion of a derivative of HT-1080 with mouse teratocarcinoma OTT6050 cells, respectively (8); cells were grown in minimal essential medium supplemented with 15% fetal calf serum, glutamine, antibiotics, and hypoxanthine-aminopterin-thymidine and, for 12-F8, 0.1 mM ouabain. The microcell hybrid cell line HDm5 (obtained from T. Lugo, Comprehensive Cancer Center, University of Southern California School of Medicine, Los Angeles) resulted from the preferential transfer by microcell fusion of human chromosome 14 in mouse 3T6 fibroblasts (17); cells were grown in a 1:1 mixture of Dulbecco minimal essential medium and Ham's F12 supplemented with 15% fetal calf serum, glutamine, antibiotics, and 500 µg of G-418 per ml (Geneticin; GIBCO).

Psoralen modification and repair analysis. Exponentially growing cells were used in all experiments to ensure active transcription of the rRNA and DHFR genes. Cell treatment, DNA isolation, and renaturing agarose gel electrophoresis (RAGE) assay were performed as described previously (31), except that mixed probes were nick translated and hybridized. The plasmid probe pR1.8 contains a 1.8-kb human genomic DHFR sequence (36), pZH4 contains a 2.2-kb CHO genomic DHFR sequence (20), and pA<sub>BB</sub> and pB<sub>ES</sub> contain 1.4-kb and 1.2-kb human genomic 28S rRNA and rRNA promoter sequences, respectively (28a).

Methods of calculation. (i) Modification of genes. The level of DNA crosslinking of gene 1 relative to that of gene 2 represents the frequency of lesions in gene 1 relative to that in gene 2. Such a ratio can be derived from the formula:

relative modification = 
$$(m_1/m_2)/(n_1/n_2)$$

with  $m_1$  and  $m_2$  corresponding to the mean number of lesions per DNA fragment in gene 1 and gene 2 and  $n_1$  and  $n_2$  corresponding to the size of the DNA fragment of gene 1 and gene 2, respectively.

A correction for differences in hybridization signal between the probes of gene 1 and gene 2 is included as follows:

relative modification =  $[(m_1/m_2)/(n_1/n_2)]/(DS_1^-/DS_2^-)$ 

with DS<sub>1</sub><sup>-</sup> and DS<sub>2</sub><sup>-</sup> corresponding to the intensities of the double-strand (DS) bands of gene 1 and gene 2, respectively, in the untreated (undenatured) samples.

The mean number of crosslinkings per fragment (m) is calculated through the simplified formula (31)

$$m_i = DS_i/[SS_i + DS_i]$$

with SS and DS equivalent to the intensities, respectively, of the denatured single-strand (SS) band and of the renatured DS band of gene i in the alkali-treated sample.

Thus, considering that  $SS_1 + DS_1 = SS_2 + DS_2$ ,

relative modification = 
$$[(DS_1/DS_2)/(n_1/n_2)]/(DS_1^-/DS_2^-)$$
 (1)

Thus, at a low level of crosslinking, the relative crosslinking frequency of gene 1 to gene 2 can be calculated from the ratio of the intensities of the renaturable crosslinked bands of gene 1 to gene 2, after corrections for differences in probe hybridization signals and in DNA fragment sizes.

(ii) Repair of genes. The efficiency of repair represents the fraction of the initial number of lesions, either crosslinkable monoadducts or interstrand crosslinks, that are removed during a particular repair period. Such fractions can be derived from the formula

relative repair = 
$$(m_o - m_t)/m_o$$

with  $m_o$  and  $m_t$  corresponding to the mean number of lesions per DNA fragment at time o and time t, respectively, after psoralen treatment.

A simplified formula can derived by using the equation  $m_i = DS_i/(SS_i + DS_i)$ :

relative repair = 
$$1 - [(DS_t/DS_o)(SS_o + DS_o)/(SS_t + DS_t)]$$
 (2)

Thus, at low levels of crosslinking, the efficiency of repair can be calculated from the relative decrease in the intensities of the renaturable crosslinked bands, after correction for differences in sample loading.

RNA isolation and slot blot hybridization. Total cellular RNA was extracted and purified by following standard procedures (19). The detection of rRNA was determined by slot blotting various amounts of total RNA of each cell line onto a Genatrans nylon support membrane. Samples were diluted in 50% (wt/vol) CsCl-0.1 M Tris (pH 7.5) and applied to the membrane by using a slot blot apparatus (Schleicher & Schuell). The wells were washed with 50% (wt/vol) CsCl before and after samples were loaded. Membranes were processed in the same manner as the Southern blots. Probes were either the pA<sub>BB</sub> with a 1.4-kb human genomic 28S rRNA sequence (11) or the pB<sub>ES</sub> with a 1.2-kb human genomic 5' rRNA sequence (28a). After being washed, membranes were exposed to Kodak XAR5 X-ray film.

### RESULTS

Strategy for measuring DNA crosslinking simultaneously in different genes. To detect HMT adducts simultaneously in the DHFR and rRNA genes, we modified our previous method of RAGE (31) in order to directly compare the level of DNA interstrand crosslinking between these two genes. The method combines the property of rapid renaturation of DNA containing covalent interstrand crosslinks with the resolution by electrophoresis in nondenaturing agarose gels of DS and SS DNA, followed by Southern blot hybridization to detect specific DNA restriction fragments. The amount of crosslinking of specific restriction fragments is determined by the distribution between noncrosslinked denatured DNA and crosslinked renatured DNA of a <sup>32</sup>P-radiolabeled probe

(31). DNA interstrand crosslinks are measured directly, whereas monoadducts are measured indirectly through their in vitro photochemical conversion into interstrand crosslinks. Here we demonstrate that by choosing an appropriate restriction enzyme to generate different fragment sizes for the respective genes and by performing simultaneous hybridization with mixed gene-specific probes, one can directly compare the levels of DNA crosslinking in different genes in the same Southern blot hybridization experiment.

Differential susceptibility of DHFR and rRNA mammalian genes to HMT modification. To establish the novel RAGE method, we first compared the levels of in vivo modification induced by HMT and UVA treatment in the rRNA genes and the DHFR genes simultaneously (Fig. 1). Because the mammalian rRNA genes are present at several hundred copies per haploid genome, we used the human cell line VA2-6A3 and the CHO cell line B11, which carry an amplified DHFR gene. Cultures of VA2-6A3 and CHO-B11 cells were exposed to HMT and UVA light, and their genomic DNAs were extracted and restricted with HindIII or KpnI, respectively. After in vitro UVA irradiation to convert monoadducts into DNA crosslinks, the DNA samples were loaded onto a neutral agarose gel as native or alkali-denatured samples. RAGE and Southern blot hybridization were performed as described previously (31), except that mixed probes were used. As expected, the native samples gave rise to two DS DNA bands, with the smaller rRNA fragment migrating faster than the DHFR fragment (Fig. 2A, lanes a and g). However, the alkali-denatured samples showed two faster migrating SS DNA bands and two DS DNA bands (Fig. 2A, lanes c and i). The persistence of DS DNA in the denatured samples is due to DNA fragments containing interstrand crosslinks (31). As shown in Fig. 2A and B, the levels of crosslinking by HMT were similar for the two genes, with the rRNA genes being modified to a lesser extent.

Two parameters can affect the covalent attachment of a chemical agent to specific cellular sequences, the total number of agent-specific binding sites on the sequence, and accessibility by the chemical agent to the sequence in its native and functional chromatin structure (31). We measured the effect of the sequences per se by treatment with HMT and UVA of purified genomic DNA from VA2-6A3 or CHO-B11 cells. As shown in Fig. 2A (lanes d to f and j to l), the rRNA sequence contains a much lower level of crosslinking than the DHFR sequence. The lower level of crosslinking in the rRNA gene relative to the DHFR gene was observed independently of the concentration of HMT. Thus, the rRNA sequence clearly contains fewer sites for the binding of HMT than does the DHFR sequence. The quantitation of the relative crosslinking levels indicates that there was approximately four- and eightfold less binding of HMT to the rRNA gene than to the DHFR gene in human and hamster genomic DNA, respectively (Fig. 2B). This lower level of modification of the rRNA gene is in contrast to the similar level of modification of the two genes in vivo (Fig. 2B). The lower level of in vitro modification of the rRNA gene relative to the DHFR gene is evidently countered in vivo by a higher accessibility of the rRNA gene to HMT modification.

Differential repair of rRNA and DHFR genes in human cells. To compare the repair of psoralen adducts in the human rRNA and DHFR genes, actively growing VA2-6A3 cells were treated with HMT and UVA under conditions facilitating the covalent binding of HMT as both monoadducts and interstrand crosslinks (32). Cells were then incu-

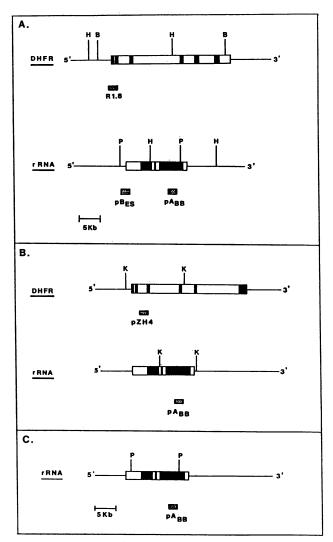
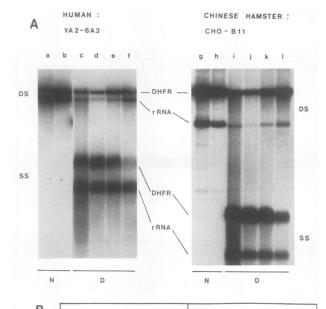


FIG. 1. Genomic maps of the functional DHFR and rRNA genes. (A) Human loci; (B) Chinese hamster loci; (C) mouse rRNA locus. Boxed areas show the regions coding for the DHFR precursor or the rRNA precursor (as marked), with the six exons of the DHFR precursor and the 5S, 18S, and 28S regions of the rRNA precursor shown as filled boxes. The locations of restriction sites are shown for the BamHI (B), HindIII (H), KpnI (K), and PvuII (P). The DHFR maps are derived from Yang et al. (36) and Milbrandt et al. (21), and the human, hamster, and mouse rRNA maps were provided by J. Sylvester, P. Wejksnora, and B. Sollner-Webb, respectively. The positions of the genomic probes used are indicated below the maps as shaded boxes. The human rRNA probe pABB, derived from a very conserved region of the 28S rRNA, was also used to detect the rRNA genes from Chinese hamster and mouse cells. In contrast, probe pB<sub>ES</sub>, derived from the 5' end of the human rRNA locus, was used as a human specific rRNA probe.

bated for various lengths of time in the presence of bromodeoxyuridine, genomic DNA was purified and *HindIII* restricted and, after the removal of the bromodeoxyuridinecontaining newly replicated DNA by cesium chloride isopycnic centrifugation, the parental DNA was used for RAGE analysis and Southern blot hybridization (Fig. 1A). Interstrand crosslinks in each sample were detected as rapidly renaturable DS DNA after alkali denaturation (Fig. 3A, lanes e to h, bottom); visual inspection of the autoradiogram



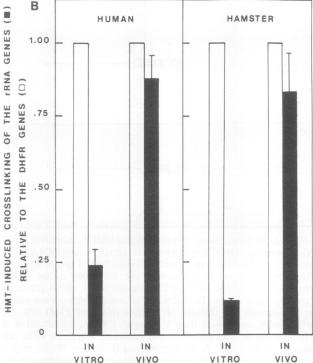


FIG. 2. Comparison of DNA crosslinking induced by HMT and UVA in the DHFR and rRNA genes of human cells or Chinese hamster cells. (A) Simultaneous detection of HMT crosslinkable adducts in the DHFR and rRNA genes by RAGE analysis. Logphase cultures of VA2-6A3 or CHO-B11 cells were either untreated or treated in vivo with 1 µg of HMT per ml and 5 min of UVA, and genomic DNA was immediately extracted, purified, and digested with HindIII (human) or KpnI (hamster). Genomic DNA treated in vivo was reirradiated for 30 min with UVA (lanes a, c, g, and i), whereas untreated genomic DNA was treated in vitro with 0 (lanes b and h), 0.01 (lanes d and j), 0.03 (lanes e and k) or 0.1 (lanes f and 1) µg of HMT per ml and 30 min of UVA. One microgram of DNA was loaded on the gel either as native DNA (N) or after alkali denaturation (D), as indicated at the bottom. After electrophoresis and Southern transfer, hybridization was performed with a mixture of <sup>32</sup>P-labeled probes, pA<sub>BB</sub> and pR1.8 for human DNA and pA<sub>BB</sub> and pZH4 for hamster DNA, respectively. Washed membranes were exposed for 30 h. Positions of probed rRNA and DHFR

reveals a difference in the rate of removal of the DNA crosslinks between the two genes, with a much slower removal from the rRNA gene than from the DHFR gene. The quantitation of this removal clearly indicates the inefficiency of repair of interstrand crosslinks from the pol I-transcribed genes compared with that of the pol II-transcribed DHFR gene (Fig. 3B). In parallel, this comparative repair analysis was applied to the HMT crosslinkable monoadducts, i.e., those monoadducts that are photoconvertible in vitro into interstrand crosslinks. In the doublet of crosslinked bands, only the upper band showed a clear decrease in the intensity with time (Fig. 3A, lanes i to 1). In other words, very little removal of monoadducts could be detected in the rRNA genes, while active removal was observed in the DHFR genes (Fig. 3B). Thus, both HMT monoadducts and interstrand crosslinks are repaired at much lower rates in the rRNA genes than in the DHFR genes in the human VA2-6A3 cells.

Differential repair in CHO cells. We also compared the repair of these same two genes in actively growing CHO-B11 cells. As shown on the autoradiogram (Fig. 4A, lanes e to h), the removal of interstrand crosslinking from the DHFR gene was extremely efficient, most crosslinks being removed within the first 8 h (Fig. 4B). In contrast, the removal of DNA crosslinking from the rRNA gene was much slower (Fig. 4A and B). The comparison of the removal of HMT-induced monoadducts was examined in the UVA reirradiated samples as was done before (Fig. 4A, lanes i to l). Again, although monoadducts were clearly removed from the DHFR gene with increasing incubation time, very little removal of these adducts occurred in the rRNA gene (Fig. 4B).

Repair of transcriptionally active and silent rRNA genes. One way to study the effect of transcription on DNA repair is to modulate the transcriptional activity of the gene. To analyze the role of pol I-mediated transcription on differential modification and repair of HMT adducts in the rRNA genes, we used the human-mouse hybrid cell line 6-F1. which carried a similar number of mouse and human rRNA genes but expressed selectively the mouse rRNA genes (Fig. 5A). Actively growing 6-F1 cells were treated with HMT and UVA as before, and genomic DNA was isolated immediately or after 48 h of incubation. The level of HMT adducts in the rRNA genes was analyzed by RAGE on PvuII-restricted DNA. The restriction fragments from the actively transcribed mouse rRNA genes migrated slightly faster than did those from the nontranscribed human rRNA genes (Fig. 5B, lanes a, b, and g). The pair of crosslinked DNA bands in the autoradiogram indicated that the initial level of modification was higher in the active mouse rRNA genes than in the silent human rRNA genes (Fig. 5B, lanes c and e). By densitometry, we estimate that transcription by the RNA pol I

restriction fragments, either DS or SS, are indicated on the sides. (B) The level of DNA crosslinking induced by HMT in the rRNA gene relative to that in the DHFR gene was calculated after the scanning densitometry of the autoradiogram (panel A) (average of three and two experiments for human and hamster cells, respectively); in vitro and in vivo conditions are as defined above for panel A. This level expresses the intensities of the DS bands in the alkalidenatured samples of the rRNA fragment relative to those of the DHFR fragment. Because corrections have been made for differences in hybridization signals and fragment sizes between the DHFR and rRNA bands, this ratio is equivalent to the relative DNA crosslinking frequency (see equation 1 in Materials and Methods).

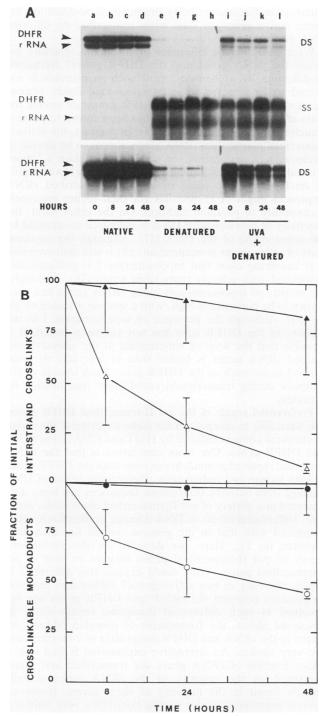


FIG. 3. Comparison of repair of HMT adducts from the human rRNA and DHFR genes. (A) Genomic DNA was isolated from growing cultures of VA2-6A3 cells treated with 1  $\mu$ g of HMT per ml and 5 min of UVA at different times after treatment (indicated in hours). After HindIII restriction and purification by CsCl equilibrium density gradient centrifugation, samples of parental DNA (1  $\mu$ g) were loaded on a nondenaturing agarose gel as either native DNA (lanes a to d), alkali-denatured DNA (lanes e to h), or alkali-denatured DNA after UVA irradiation for 30 min (lanes i to l). After electrophoresis, Southern transfer, and hybridization with a mixture of pA<sub>BB</sub> and pR1.8 probes, the filter was exposed for 20 h without (upper part of panel) or with (lower part of panel) an intensifying screen. Positions of the probed fragment, either DHFR or rRNA and either DS or SS, are indicated on the sides. (B) The

increased the susceptibility to HMT addition by approximately a factor of five. However, the comparison of band intensities between 0 and 48 h did not show any significant change in the DNA crosslinking level for either interstrand crosslinks or monoadducts (Fig. 5B, lanes c to f). In other words, although the actively transcribed mouse rRNA genes are clearly more accessible to chemical modification by HMT, their repair inefficiency is not affected relative to that of the nontranscribed human rRNA genes.

#### DISCUSSION

We have demonstrated that the efficiency of repair of HMT adducts in rRNA genes is distinctly different from that in DHFR genes in both human and hamster cells, with much more efficient repair of DHFR genes. We also observed a differential effect on the binding of HMT adducts; a specific increase in susceptibility to chemical modification in active rRNA genes is demonstrated in a human-mouse hybrid cell line that carries actively transcribed mouse and nontranscribed human rRNA genes. We conclude that both the levels of addition and the efficiency of repair of HMT adducts vary with the type of RNA polymerase utilized for transcribing the respective genes.

Preferential modification of the pol I-transcribed rRNA genes. By combining RAGE analysis (31) with simultaneous hybridization with several probes and appropriate enzymatic restriction, we were able to compare the levels of psoralen modification of the endogenous pol I-transcribed rRNA genes and the pol II-transcribed DHFR genes after in vitro or in vivo treatment. A primary conclusion is that the rRNA genes are more susceptible to psoralen modification than are the DHFR genes. Two different experiments support this conclusion. (i) Although there are fewer binding sites for psoralen in the primary sequence of rRNA genes than in that of DHFR genes, the levels of modification of the rRNA and DHFR genes after in vivo treatment are very similar. Thus, the rRNA genes in their native chromatin structure are more accessible to psoralen modification than are the DHFR genes. (ii) In interspecies hybrid cells, the actively transcribed rRNA genes are more susceptible to psoralen modification than are the nontranscribed rRNA genes. Thus, a difference in chromatin structure between transcribed and nontranscribed rRNA genes is responsible for the preferential modification by psoralen of the active genes. Heterogeneous distribution of DNA lesions caused by different chemical carcinogens has previously been reported (reviewed in references 2 and 4). Such lesions have been found to predominate in the transcribing regions of chromatin, including rRNA genes (2, 7, 22). The present study reveals

initial fraction of HMT adducts, either interstrand crosslinks (triangles) or crosslinkable monoadducts (circles), remaining in the HindIII fragment of the rRNA genes (filled symbols) and of the DHFR genes (open symbols) after the indicated repair period was calculated after densitometric analysis of the autoradiogram (panel A). This fraction expresses the ratio of the intensities of the DS bands at the repair time t to those at the initial time, which are measured in the alkali-denatured samples for each gene. Because correction has been made for differences in sample loading, this ratio is equivalent to the fraction of unrepaired lesions (see equation 2 in Materials and Methods). The upper and lower exposures of Fig. 3A were used to quantitate monoadducts (lanes i to l) or interstrand crosslinks (lanes e to h), respectively (average of four experiments). Error bars refer to standard deviations.

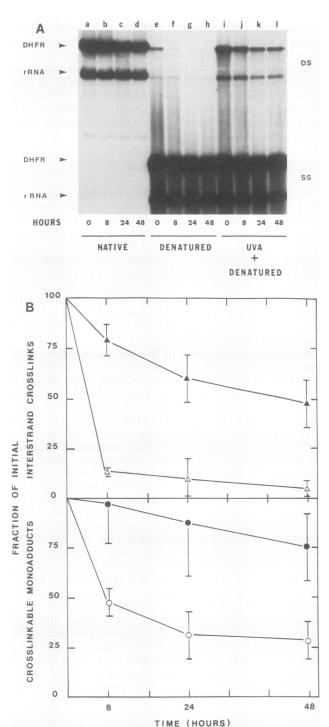


FIG. 4. Comparison of repair of HMT adducts from hamster rRNA and DHFR genes. (A) Genomic DNA was isolated from growing cultures of CHO-B11 cells treated with 1 µg of HMT per ml and 5 min of UVA at different times after treatment (indicated in hours). After KpnI restriction and purification of parental DNA as described in the legend to Fig. 3, DNA samples (1 µg) were loaded on a nondenaturing agarose gel as either native DNA (lanes a to d), alkali-denatured DNA (lanes e to h), or alkali-denatured DNA after UVA irradiation for 30 min (lanes i to l). After electrophoresis, Southern transfer, and hybridization with a mixture of pA<sub>BB</sub> and pZH4 probes, the filter was exposed for 18 h with an intensifying screen. Positions of the probed fragments, either DHFR or rRNA and either DS or SS, are indicated on the sides. (B) The initial fraction of HMT adducts, either interstrand crosslinks (triangles) or

variation in the susceptibility to chemical modification between active genes, at least when transcribed by different RNA polymerases.

What could be the difference in chromatin structure between the rRNA genes and the DHFR genes? Intragenic modification by a chemical agent such as psoralen is expected to be directed to internucleosomal linker regions (reviewed in reference 4). The DHFR genes are among the class of housekeeping genes that has been shown to possess a nucleosomal organization (16, 25). In contrast, the actively transcribed mammalian rRNA genes appear to be devoid of nucleosomal organization, or at least to contain a "more relaxed" nucleosomal structure (7, 27). Thus, a lower degree of nucleosomal packaging of actively transcribed rRNA sequences might be responsible for their higher intragenic accessibility to psoralen addition. On the other hand, the reactivity of psoralen with DNA is increased severalfold by the supercoiling of the DNA (37). Although the torsional state of active genes in mammalian cells is still controversial, it is becoming clear that topoisomerase I is preferentially associated with actively transcribed genes (10). Strikingly, the density of topoisomerases along rRNA genes has been shown to be particularly high, with a spacing distance of 200 bp (39). Although the presence of topoisomerase I in the vicinity of the DHFR gene has not yet been tested, it is possible that the level of supercoiling in the actively transcribed rRNA genes is higher than in less actively transcribed genes such as the DHFR gene. Such torsion of the template during transcription could favor modification by psoralen.

Preferential repair of the pol II-transcribed DHFR genes. We were able to compare in the same experiment the repair of chemical adducts induced by HMT and UVA in the rRNA and DHFR genes. Our main conclusion is that the rRNA genes are repaired at much lower rates than the DHFR genes in both human and rodent cells. Preferential repair of DNA damage from actively transcribed sequences has been documented in a variety of pol II-transcribed mammalian genes (26). Differential repair of DNA damage in the rRNA genes compared with that in the genome overall has also been reported (6, 13). Here, we demonstrate the preferential repair of pol II-transcribed genes relative to that of pol I-transcribed genes. What could explain this difference in repair efficiency in two active genes? Differences in lesion frequencies between the rRNA and DHFR genes are not involved in such differential intragenic repair since, as discussed above, the frequencies of psoralen adducts induced in the rRNA and DHFR genes after in vivo treatment are very similar. An alternative explanation is that only a minor fraction of rRNA genes are transcribed and repair measured on the majority of the rRNA genes actually reflects repair in the majority of silent genes. However, several arguments render such a possibility very unlikely. First, because the level of rRNA transcription is highly dependent on cell growth, we used exponentially growing cells to maximize the fraction of rRNA loci which were

crosslinkable monoadducts (circles), remaining in the *KpnI* fragment of the rRNA genes (filled symbols) and of the DHFR genes (open symbols) after the indicated repair period was calculated after densitometric analysis of the autoradiogram (panel A) as indicated in the legend to Fig. 3B (average of two experiments). Overexposed autoradiograms were used to quantitate interstrand crosslinks (lanes e to h).

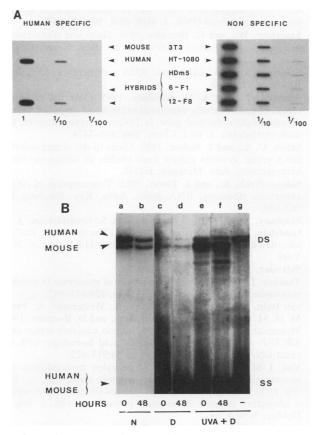


FIG. 5. Comparison of repair of HMT adducts from the active mouse genes and silent human rRNA genes in cell hybrids. (A) Slot blot analysis of the pattern of rRNA transcription in human-mouse cell hybrids. Total cellular RNA was isolated by guanidinium chloride extraction from growing cultures of three human-mouse hybrid clones, two derived from synkaryon fusion (6-F1 and 12-F8) and one derived from the transfer by microcell fusion of the human chromosome 14 into mouse cells (HDm5); as a control, total cellular RNA was isolated from mouse (3T3) and human (HT-1080) fibroblasts. Equivalent amounts of cellular RNA (1 µg, 0.1 µg, and 0.01 μg) were transferred in duplicate to two nitrocellulose membranes to allow parallel hybridization with the human specific probe pB<sub>ES</sub> (left panel) or the human nonspecific probe pA<sub>BB</sub> (right panel). In agreement with previous somatic cell hybridization experiments, fusion of human and mouse cell lines led to the suppression of the rRNA transcription of one species, the so-called nucleolar dominance phenomenon (8). (B) Growing cultures of the human-mouse hybrid cell line 6-F1 were treated with 1 µg of HMT per ml and 5 min of UVA, and genomic DNA was isolated either immediately or after 48 h of posttreatment incubation (indicated in hours). After PvuII restriction and purification of parental DNA as described in the legend to Fig. 3, DNA samples (3 µg) were loaded on a nondenaturing agarose gel as either native DNA (lanes a and b), alkalidenatured DNA (lanes c and d), or alkali-denatured DNA after UVA irradiation for 30 min (lanes e and f). In parallel, PvuII-restricted human genomic DNA extracted from VA2-6A3 cells was loaded as native DNA (lane g). After electrophoresis, Southern transfer, and hybridization with the probe pA<sub>BB</sub>, the filter was exposed for 38 h with an intensifying screen. Positions of the probed rRNA fragments, either human or mouse and either DS or SS, are indicated on the sides.

actively transcribing; in rapidly growing cells, it has been shown that most rRNA genes are effectively transcribed (7, 9). Second, in the interspecies hybrids, active rRNA transcription increased the level of psoralen modification severalfold but did not change the repair inefficiency of the genes. Third, we have observed that transcription of rRNA genes specifically increased their susceptibility to chemical modification by psoralen (see above). Because of the level of such increase (four- and sixfold in human and hamster cells, respectively), 75 to 85% of the psoralen adducts in rRNA genes must be bound to actively transcribed rRNA genes. Thus, psoralen adducts that are preferentially localized in transcribed rRNA genes are not selectively removed.

The much lower rate of repair of psoralen adducts in the rRNA genes indicates that preferential repair of these adducts does not occur in these genes. What could be the reason for the apparent absence of coupling between transcription and repair in the rRNA genes? One possibility could involve the chromatin structure in which these genes are embedded. rRNA genes form a distinct category of housekeeping genes because of their organization into a particular chromosomal substructure, the nucleolus (reviewed in reference 14). Because of the tight packing of the nucleolar chromatin around the rRNA genes, access to damaged rRNA genes by the repair complex could be restricted. A parallel could be drawn with the inefficient repair of psoralen adducts in the highly condensed chromatin of the tandemly repeated DNA alpha sequences of monkey cells (40). However, this interpretation must also accommodate the predicted accessibility of rRNA genes to the transcription machinery, i.e., pol I and cofactors. Another possibility is that the repair complex has access to the rRNA genes but that transcription mediated by pol I, in contrast to that mediated by pol II, does not facilitate preferential removal of the damage from their templates. Further study should help to distinguish between these alternatives.

The different rates of repair of psoralen adducts for DHFR and rRNA genes occur in both human and hamster cells; in addition, differential repair is observed for two structurally distinct DNA adducts, i.e., intrastrand monoadducts and interstrand crosslinks. Apparently, these results extend the original observation of differential gene repair made with UV-induced DNA damage (reviewed in 26) to chemical DNA damage. However, in contrast to the faster repair of UV-induced cyclobutane pyrimidine dimers in human cells than in hamster cells, psoralen adducts are repaired faster in hamster than in human cells (Fig. 3 and 4). This suggests that, while rodent cells are more prone to differential repair of UV damage, human cells may be more prone to differential repair of chemical damage. Additional comparative studies of differential repair of DNA adducts in rodent and primate genes will be necessary to determine the validity of such a general assertion.

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